CLAIMS

What is claimed is:

1. A recombinant replicon nucleic acid comprising:
a first nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;
at least one second nucleic acid sequence encoding an alphavirus nonstructural protein;
at least one alphavirus subgenomic promoter;
at least one IRES element;
at least one heterologous nucleic acid; and
a third nucleic acid encoding a 3' alphavirus replication recognition sequence.

2. The nucleic acid of claim 1, wherein the second nucleic acid is a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp2, nsp3 and nsp4.

3. The nucleic acid of claim 1, comprising an original second nucleic acid sequence that is a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp 2 and nsp3 and further comprising an additional second nucleic acid encoding alphavirus nonstructural protein nsp4 that is not contiguous with the original second nucleic acid.

4. The nucleic acid of claim 1, wherein the IRES element is selected from the group consisting of cellular IRESs, plant IRESs, mammalian virus IRESs, synthetic IRESs and insect virus IRESs.

5. The nucleic acid of claim 1, wherein the alphavirus subgenomic promoter is a minimal or modified alphavirus subgenomic promoter.

6. The nucleic acid of claim 1, wherein the heterologous nucleic acid

encodes a protein.

7. The nucleic acid of claim 1, wherein the heterologous nucleic acid is an antisense sequence.

8. The nucleic acid of claim 1, wherein the heterologous nucleic acid encodes a ribozyme.

9. The nucleic acid of claim 1, wherein the heterologous nucleic acid encodes an alphavirus structural protein.

10. The nucleic acid of claim 9, wherein the alphavirus structural protein is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

11. The nucleic acid of claim 1, wherein the first nucleic acid sequence encoding a 5' alphavirus replication recognition sequence is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

12. The nucleic acid of claim 1, wherein the second nucleic acid sequence encoding an alphavirus nonstructural protein is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

13. The nucleic acid of claim 1, wherein the alphavirus subgenomic promoter is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

14. The nucleic acid of claim 1, wherein the third nucleic acid encoding a 3' alphavirus replication recognition sequence is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

15. The nucleic acid of claim 1, wherein the IRES element directs the translation of the gene product encoded by the heterologous nucleic acid, such that at least 80% of the translation of the gene product encoded by the heterologous nucleic acid is controlled by the activity of the IRES element.

16. The nucleic acid of claim 1, wherein the IRES elements directs the translation of the gene product encoded by the heterologous nucleic acid such that at least 10% of the translation of the gene product encoded by the heterologous nucleic acid is controlled by the activity of the IRES element.

17. The nucleic acid of claim 1, wherein the nucleic acid is RNA.

18. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

19. The nucleic acid of claim 1, further comprising a spacer nucleic acid sequence located upstream of the IRES element.

20. The nucleic acid of claim 19, wherein the spacer nucleic acid sequence is at least 30 nucleotides in length.

21. The nucleic acid of claim 19, wherein the spacer nucleic acid sequence is between 25 and 7500 nucleotides in length.

22. The nucleic acid of claim 19, wherein the spacer nucleic acid sequence is between 150 and 1000 nucleotides in length.

23. A population of infectious, defective, alphavirus particles, wherein each particle comprises the nucleic acid of claim 1, and the population has no detectable replication-competent virus, as measured by passage on cell culture.

24. A population of infectious, defective, alphavirus particles, wherein each particle comprises the nucleic acid of claim 19, and the population has no detectable replication-competent virus, as measured by passage on cell culture.

25. A pharmaceutical composition comprising the population of claim 23 in a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising the population of claim 24 in a pharmaceutically acceptable carrier.

27. An alphavirus particle comprising a recombinant nucleic acid according to claim 1.

28. An alphavirus particle comprising a recombinant nucleic acid according to claim 19.

29. The alphavirus particle of claim 27, comprising an attenuating mutation.

30. The alphavirus particle of claim 29, comprising an attenuating mutation.

31. The nucleic acid of claim 1, comprising an attenuating mutation.

32. The nucleic acid of claim 19, comprising an attenuating mutation.

33. A population of infectious, defective, alphavirus particles, wherein each particle comprises the nucleic acid of claim 1.

34. A population of infectious, defective, alphavirus particles, wherein each particle comprises the nucleic acid of claim 19.

35. A composition comprising the population of claim 32, in a pharmaceutically acceptable carrier.

36. A composition comprising the population of claim 33, in a pharmaceutically acceptable carrier.

37. A method of making infectious, defective alphavirus particles, comprising:
a) introducing into a cell the following:

(i) a recombinant nucleic acid according to claim 1,and

(ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the one or more helper nucleic acids produce all of the alphavirus structural proteins, and

b) producing said alphavirus particles in the cell.

37. The method of claim 36, wherein the recombinant nucleic acid comprises at least one heterologous nucleic acid encoding an alphavirus structural protein.

38. The method of claim 36, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, a nucleic acid encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

39. The method of claim 36, wherein the helper nucleic acid is a recombinant nucleic acid comprising a promoter and nucleotide sequences encoding one or more alphavirus structural proteins.

40. The method of claim 38, wherein the helper nucleic acid is DNA.

41. The method of claim 40, wherein the promoter is a CMV promoter.

42. The method of claim 40, wherein the helper nucleic acid comprises nucleotide sequences encoding all of the alphavirus structural proteins.

43. The method of claim 36, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an IRES element, a nucleic acid encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

44. The method of claim 36, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, an IRES element, a nucleic acid encoding one or

more alphavirus structural proteins and a 3' alphavirus replication recognition sequence.

45. A method of making infectious, defective alphavirus particles, comprising:
a) introducing into a cell the following:

i) an alphavirus replicon RNA comprising a 5' alphavirus replication recognition sequence, nucleic acid sequence(s)encoding alphavirus nonstructural proteins, an alphavirus subgenomic promoter, a heterologous nucleic acid sequence and a 3' alphavirus replication recognition sequence; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, an IRES element, a nucleic acid encoding one or more alphavirus structural proteins and a 3' alphavirus replication recognition sequence, whereby all of the alphavirus structural proteins are produced in the cell; and
b) producing said alphavirus particles in the cell.

46. A method of making infectious, defective alphavirus particles, comprising:
a) introducing into a cell the following:

i) an alphavirus replicon RNA comprising a 5' alphavirus replication recognition sequence, nucleic acid sequence(s) encoding alphavirus nonstructural proteins, at least one alphavirus subgenomic promoter, at least one IRES element, at least one heterologous nucleic acid sequence and a 3' alphavirus replication recognition sequence; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, an IRES element, a nucleic acid encoding one or more alphavirus structural proteins and a 3' alphavirus replication recognition sequence, whereby all of the alphavirus structural proteins are produced in the cell; and
b) producing said alphavirus particles in the cell.

47. A recombinant nucleic acid comprising 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, an IRES element, a nucleic acid encoding one or more alphavirus structural proteins and a 3' alphavirus replication recognition sequence.

48. A cell comprising the nucleic acid of claim 47.

49. The nucleic acid of claim 1, further comprising an alphavirus packaging signal.

50. The nucleic acid of claim 1, further comprising a spacer nucleic acid sequence upstream of an IRES element.

51. The nucleic acid of claim 47, further comprising a spacer nucleic acid sequence upstream of an IRES element.

52. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the population of claim 23.

53. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the population of claim 24.

54. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the population of claim 33.

55. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the population of claim 34.

56. A recombinant nucleic acid comprising:
a promoter that directs transcription of a nucleic acid;
an IRES element; and
a coding sequence,
wherein the IRES element is operably located such that translation of the coding sequence is via a cap-independent mechanism directed by the IRES element.

57. A recombinant nucleic acid comprising:
a first nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;

at least one second nucleic acid sequence encoding an alphavirus nonstructural protein;

a first alphavirus subgenomic promoter;

a first IRES element;

a first heterologous nucleic acid;

a second alphavirus subgenomic promoter;

a second IRES element;

a third nucleic acid encoding a 3' alphavirus replication recognition sequence.

58. The nucleic acid of claim 57, further comprising an alphavirus packaging signal.

59. The nucleic acid of claim 57, further comprising a spacer nucleic acid sequence upstream of an IRES element.